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Cell–Cell Recognition: Specific Binding of *Microciona* Sponge Aggregation Factor to Homotypic Cells and the Role of Calcium Ions†

James E. Jumblatt,‡ Verena Schlup, and Max M. Burger*

ABSTRACT: Previous studies have established that the mechanism of species-specific sponge cell reaggregation in vitro involves at least three components: a species-specific aggregation factor, its cell surface receptors, and Ca^{2+} ions. Here we report about the binding of *Microciona* ^{125}I-labeled aggregation factor to cells and to other molecules of aggregation factor coupled to agarose beads, as well as the role of divalent cations in both factor–cell and factor–factor interactions. Our results indicate that cell aggregation is a two-step process, involving (1) Ca^{2+}-independent species-specific binding of the 21×10^6 dalton aggregation factor to cellular receptors and (2) formation of Ca^{2+}-dependent linkages between factor molecules on adjacent cells. Quantitation of binding suggests that less than 5% of the cell-associated aggregation factor in vivo is required for cell–cell adhesion in the in vitro assay. Alternative biological functions for the aggregation factor are discussed.

Selective cell–cell adhesion is thought to play an important role during embryonic tissue formation in vertebrates, although the molecular and regulatory mechanisms are poorly understood (Moscona, 1962; Steinberg, 1970). The species-specific reaggregation of dissociated sponge cells, described by Wilson in 1907, has proven to be a useful experimental model for both intercellular recognition and adhesion (Wilson, 1907; Humphreys, 1963; Van de Vyver, 1975). When dissociated sponge cells from some different species are mixed in seawater, cells reaggregate species specifically and in some cases form reconstituted sponges. The selective reaggregation of cells is mediated by specific surface components, termed aggregation factors (AF's),[1] which have been isolated from a variety of sponge species (Humphreys, 1963; Moscona, 1968; Müller & Zahn, 1973; Müller et al., 1978). The aggregation factors are removed by washing cells in Ca^{2+}- and Mg^{2+}-free seawater (CMF-SW) and must be added back to washed cells for reaggregation in normal, divalent cation containing seawater. Although some aggregation factors have been purified and biochemically characterized, the molecular basis for their specificities, requirements for divalent cations, and mode of action to promote cell–cell adhesion are unclear (Müller & Zahn, 1973; Müller et al., 1978; Henkart et al., 1973).

We have investigated the mechanism of factor-promoted cell reaggregation in the marine sponge *Microciona prolifera* (Weinbaum & Burger, 1973; Turner & Burger, 1973). The *Microciona* aggregation factor has been characterized as a 2.1×10^7 dalton fibrous protein–polysaccharide complex having an unusual "sunburst" appearance in electron micrographs and containing several thousand binding sites for Ca^{2+} (Henkart et al., 1973; Cauldwell et al., 1973; Humphreys et al., 1977). A complex involvement of Ca^{2+} in the structure and function of aggregation factor was indicated by the dissociation of factor from cells in divalent cation free seawater, the irreversible inactivation and physical dissociation of the factor molecule into subunits by EDTA treatment, and the tendency of the factor to form gels at elevated Ca^{2+} concentrations (Henkart et al., 1973; Cauldwell et al., 1973). These findings suggested possible roles for Ca^{2+} in factor stability, binding of factor to cells, and interaction between factor molecules, all of which may be essential for cell aggregation.

In this study, we have characterized the binding of radioiodinated *Microciona* aggregation factor to cellular receptors, using fixed and live cells from different sponge species. The Ca^{2+}-dependent, functional association between factor molecules was analyzed in a model aggregation system by using aggregation factor conjugated to agarose beads (Weinbaum & Burger, 1973). A two-stage mechanism for cell–cell adhesion is proposed, and the biological function of aggregation factor is discussed.

Materials and Methods

Sponges. Live specimens of *M. prolifera*, *Haliclona oculata*, *Cliona celata*, and *Halichondria panicea* were collected in the Woods Hole area during the months of July through September. Sponges were routinely used on the day of collection but could be maintained at ambient temperatures in tanks of running seawater as long as 1 week.

Buffers. Bicarbonate-buffered artificial seawater (MBL-SW) was prepared according to the Marine Biological Laboratory formula (Humphreys, 1963; Cavenough, 1956). Ca^{2+}- and Mg^{2+}-free seawater (CMF-SW) was made as described by Humphreys (1963). Isolated aggregation factor was stored in 0.02 M Tris-buffered CMF-SW (pH 7.2) containing 2 mM $CaCl_2$ (CaCMFT) and 0.1% NaN_3. Both CaCMFT and MBL-SW buffered with 0.02 M Tris (MBLT) to pH 7.2 were used to wash cells after incubation with [^{125}I]AF in the binding assay.

Dissociated Sponge Cells. Suspensions of mechanically dissociated sponge cells were obtained by gentle squeezing of sponge fragments through fine-mesh silk bolting cloth into cold (4 °C) bicarbonate-buffered MBL-SW. Chemically dissociated cells were prepared by the procedures of Humphreys (1963). Briefly, sponge fragments were dissociated into single cells in cold bicarbonate-buffered CMF-SW and incubated in the same buffer for several hours, followed by washing to remove most of the surface-associated aggregation factor.

† From the Department of Biochemistry, Biocenter, University of Basel, 4056 Basel, Switzerland. *Received May 31, 1979; revised manuscript received October 16, 1979.* This work was support by the Swiss National Foundation, Grant No. 3.721-076.

‡ Present address: Department of Biochemistry and Pharmacology, Tufts University School of Medicine, Boston, MA 02111.

[1] Abbreviations used: AF, aggregation factor; CMF-SW, Ca^{2+}- and Mg^{2+}-free seawater; CaCMFT, Ca^{2+}- and Mg^{2+}-free seawater that was Tris buffered and where Ca^{2+} was restored to 2 mM; MBL-SW, artificial seawater prepared according to the formula of Cavenough (1956) at the Marine Biological Laboratory, Woods Hole; MBLT, identical with MBL-SW except for being buffered with Tris; Cl_3AcOH, trichloroacetic acid; BSA, bovine serum albumin.

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Fixation of sponge cells with 1% glutaraldehyde was performed as described previously (Jumblatt et al., 1977).

Isolation, Purification, and Assay of Aggregation Factor. *Microciona* aggregation factor (AF) was extracted and purified at 4 °C according to the methods of Humphreys, with slight modifications (Humphreys, 1963; Humphreys et al., 1975). The supernatant obtained from chemically dissociated cells (see above) was buffered with 0.02 M Tris, pH 7.2, and clarified by sequential centrifugations at 1000*g* for 10 min and 22000*g* for 30 min. The supernatant was adjusted to 30 mM $CaCl_2$ and stirred overnight, causing the aggregation factor to form a gel which was removed by centrifugation at 10000*g* for 20 min. The gel containing the AF was dissolved in a small volume of CaCMFT and then centrifuged at 105000*g* for 90 min. The clear, yellowish pellet was dissolved in 5–10 mL of CaCMFT and fractionated in the same buffer on a 3 × 75 cm column of Sepharose 2B (Pharmacia). Active column fractions, emerging in the void volume, were pooled and centrifuged at 105000*g* for 90 min to pellet the factor. The transparent, colorless pellet was dissolved in CaCMFT with 0.01% NaN_3 and could be stored for several months without detectable loss of activity.

The specific activity of purified aggregation factor in promoting cell aggregation was 1.25–1.8 units/μg of protein (Lowry et al., 1951) as assayed by the method of Henkart et al. (1973). Briefly, serial twofold dilutions of AF were incubated with cells on a rotary shaker for 20 min at 23 °C. Following the incubation, the macroscopic end point—i.e., the maximum dilution of AF causing cells to aggregate—was determined by inspection and expressed as units per milliliter. Thus, AF with an activity of 64 units/mL contains 64 times the minimum AF concentration (1 unit/mL) needed to aggregate cells in the standard assay.

Radioiodination of Aggregation Factor. Purified aggregation factor was labeled with ^{125}I by using a chloramine-T procedure. All steps were performed at 4 °C. Five milliliters of aggregation factor (128–256 units/mL) in CaCMFT was mixed with 2.5 mCi of $Na^{125}I$ (New England Nuclear, carrier free) and 125 μL of 10 mM chloramine-T dissolved in H_2O. The reaction was stopped after 5 min by addition of 125 μL of 20 mM sodium metabisulfite. Following dialysis against 2000 volumes of CaCMET containing 50 mM NaI, the labeled aggregation factor was sedimented on a gradient of 10–30% sucrose in CaCMFT (39000 rpm, 170 min, Beckman SW 41 rotor) and gradient fractions were assayed for radioactivity and aggregation-promoting activity. Activity fractions, which coincided with a peak of radioactivity, were pooled, dialyzed against CaCMFT, and concentrated for binding assays. The specific radioactivity of the labeled aggregation factor preparations varied from 0.178 to 0.238 μCi/μg of protein or (1.87–2.5) × 10^6 Ci/mol of 2.1 × 10^7 dalton aggregation factor monomer containing 50% polypeptide by weight (Jumblatt et al., 1977). The iodination resulted in no detectable loss of factor activity. More than 90% of the incorporated radioactivity was precipitable with 10% Cl_3AcOH at 4 °C, and more than 80% coprecipitated with unlabeled aggregation factor after 2 h of incubation in 30 mM $CaCl_2$ (data not shown).

Conjugation of Aggregation Factor to Agarose Beads. Purified aggregation factor was coupled covalently to commercially prepared CNBr-activated Sepharose 4B (Pharmacia) beads according to the instructions supplied by the manufacturer. All steps were performed at 4 °C. Two milliliters of packed, activated beads was suspended in 5 mL of coupling buffer (CMF-SW, 2 mM $CaCl_2$, and 0.1 M $NaCHO_3$), quickly mixed with an equal volume of aggregation factor (512 units/mL) in the same buffer, and incubated with gentle stirring for 16 h. Bovine albumin conjugated beads were prepared identically, using 1 mg/mL bovine serum albumin (BSA, Sigma Type I) in place of aggregation factor. Following conjugation, beads were washed on a glass filter with more than 50 volumes of CaCMFT, treated with glycine (0.1 M in CaCMFT) for 2 h, and then washed extensively with CaCMFT. The beads were stored at 4 °C in CaCMFT containing 0.01% NaN_3.

Aggregation Assay. The end point dilution assay of factor-promoted aggregation of cells or factor-coated beads was performed in multiwell Linbro plates as described previously (Humphreys, 1963). The progressive increase in the macroscopically visible end point was recorded at 2-min intervals throughout the 20-min assay period (Burger & Jumblatt, 1977) to follow the kinetics of aggregation.

[^{125}I]AF Binding Assay. Dilutions of labeled AF were incubated with cells or factor-coupled beads for 20 min under the standard aggregation assay conditions described above. Following incubation, 0.2-mL aliquots were removed, layered on a 1-mL cushion of 10% sucrose, 0.1% BSA, and CaCMFT or MBLT in a tapered Eppendorf centrifuge tube, and centrifuged for 5 min at 1000*g*. The supernatant was aspirated, and the tip of the tube containing the cell pellet was cut off with a razor blade and counted in a Packard Tri-Carb γ counter. In control experiments (not shown), the sucrose–BSA mixture had no effect on the dissociation of bound aggregation factor from cells or factor beads. The radioactivity in controls without cells or beads (less than 5% of input) was subtracted from the experimental values to determine specific binding. The number of labeled AF molecules bound per cell was calculated from the number of cells in the assay (4 × 10^6), the specific radioactivity of the bound aggregation factor [(1.87–2.5) × 10^6 Ci/mol], and Avogadro's number.

Baseplate Isolation. Isolation and partial purification of the receptor-like baseplate component from *Microciona* cells were performed as described previously (Weinbaum & Burger, 1973; Jumblatt et al., 1977). The inhibitory supernatant from hypotonically treated cells was further fractionated by differential centrifugation, ammonium sulfate precipitation, and Sephadex G-150 gel filtration. The baseplate preparation used in this study was purified approximately 60-fold over starting material, based on assays of the inhibition of AF-promoted cell aggregation (Jumblatt et al., 1977; Burger & Jumblatt, 1977).

Results

Specific Binding of ^{125}I-Labeled Aggregation Factor to Microciona Cells. Figure 1 shows the concentration-dependent binding of [^{125}I]AF to fixed homotypic cells in Ca^{2+}-containing seawater (MBLT) under standard aggregation assay conditions. In preliminary studies, similar binding curves were obtained by using glutaraldehyde-fixed or live cells, as expected from the unchanged aggregation characteristics of fixed cells in response to added AF (Burger & Jumblatt, 1977). The [^{125}I]AF binding curve appears to be nonsaturable and suggests possible cooperativity or additional sites at AF concentrations above 8 units/mL. However, such interpretations of the shape of the binding curve are complicated by the unknown effect of cell aggregation on AF binding. Nevertheless, the use of glutaraldehyde-fixed cells rules out any significant uptake of [^{125}I]AF by endocytosis or a dynamic rearrangement of surface receptors as explanations of nonsaturable or cooperative binding kinetics.

Table I shows the species specificity of [^{125}I]AF binding at 1 unit/mL, the lowest AF concentration capable of aggregating

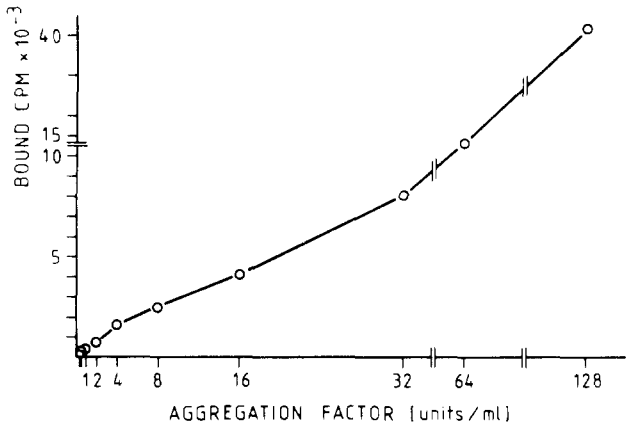

FIGURE 1: Binding of *Microciona* ^{125}I-labeled aggregation factor to dissociated cells. Glutaraldehyde-fixed, chemically dissociated *Microciona* cells were incubated with various concentrations of ^{125}I-labeled aggregation factor (1.9 × 10^6 Ci/mol) in Ca^{2+}-containing MBLT. (See Materials and Methods for details of binding assay.) After 20 min of incubation, 0.2-mL aliquots of cells with bound [^{125}I]AF were separated from unbound AF by centrifugation, and cell-bound radioactivity was determined.

Table I: Binding Specificity of *Microciona* ^{125}I-Labeled Aggregation Factor to Live and Fixed Cells in Ca^{2+}-Containing Seawater

sponge cells	amount of [^{125}I]AF bound[a] molecules/cell	(molecules/mg of cell protein) × 10^{-10}
M. prolifera		
live	516	1.28
fixed	407	1.36
H. panicea		
live	18	0.11
fixed	33	0.19
C. celata		
live	28	0.11
fixed	32	0.12

[a] Live or glutaraldehyde-fixed, chemically dissociated sponge cells (10^7/mL) were incubated with *Microciona* [^{125}I]AF (1 unit/mL; 1.92 × 10^6 Ci/mol) for 20 min at room temperature under standard assay conditions. Following incubation, cells were centrifuged and counted to determine bound [^{125}I]AF (see Materials and Methods). The number of 2.1 × 10^7 dalton AF molecules bound in each case was calculated from the specific radioactivity (1.92 × 10^6 Ci/mol) and Avogadro's number and was divided by the number of cells added (4 × 10^6) or the corresponding amount of cell protein in the assay (Lowry et al., 1951). The values shown are the averages of duplicate determinations.

homotypic cells. *Microciona* cells bound 400–600 AF molecules/cell, whereas *Cliona* or *Halichondria* cells (which did not aggregate) bound less than 40 molecules/cell. These differences in binding were seen regardless of the reference parameter (cell protein or cell number) and were unchanged by glutaraldehyde fixation of cells.

Effect of Ca^{2+} on Binding of Microciona ^{125}I-Labeled Aggregation Factor. Figure 2 shows the binding of [^{125}I]AF to cells at various Ca^{2+} concentrations, and the Ca^{2+} dependence of AF-promoted cell aggregation is included for comparison. At an [^{125}I]AF concentration of 1 unit/mL, no significant differences in AF binding were observed between 0.1 and 30 mM Ca^{2+}, although cell aggregation occurred only above 10 mM Ca^{2+}. This result was unexpected, since AF is removed from freshly dissociated cells by incubation in Ca^{2+}- and Mg^{2+}-free seawater and was therefore assumed to require Ca^{2+} for binding (Henkart et al., 1973). An additional experiment was performed to test whether the 400–600 AF molecules

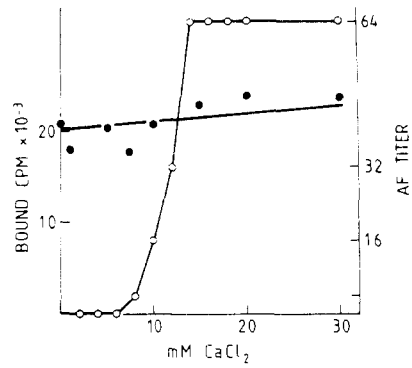

FIGURE 2: Effect of Cd^{2+} on [^{125}I]AF binding to cells and on AF-mediated cell aggregation. ^{125}I-Labeled aggregation factor (1 unit/mL, 2.1 × 10^6 Ci/mol) was incubated with 4 × 10^6 glutaraldehyde-fixed, chemically dissociated cells in CMFT with added $CaCl_2$ at the concentrations shown. After 20 min of incubation, binding of [^{125}I]AF to cells was determined as described under Materials and Methods. The Ca^{2+} dependence of AF-promoted cell aggregation was determined separately. Unlabeled AF (64 units/mL) was serially diluted into wells containing 0.2-mL volumes of Ca^{2+}-supplemented CMFT at twice the Ca^{2+} concentrations shown. The assay was started by adding fixed, chemically dissociated cells in CMFT, and the end point at each Ca^{2+} concentration was read after 20 min as described under Material and Methods. (●) Counts per minute of [^{125}I]AF bound; (○) AF titer.

bound per cell at the lowest Ca^{2+} concentration were sufficient for cell aggregation at the optimal Ca^{2+} concentration or whether additional AF molecules were required. Cells were incubated with 1 unit/mL [^{125}I]AF in divalent cation free seawater (CMFT) and were washed by centrifugation in 10 volumes of the same buffer to remove unbound AF. They were then incubated for 20 min in divalent cation containing seawater (MBLT) under standard assay conditions. The amount of aggregation observed in the second incubation was equivalent to controls incubated with 1 unit/mL AF in MBLT for an identical assay period. This experiment was interpreted to show that the bound AF molecules are converted to a functional state at the calcium concentrations optimal for cell aggregation.

A model system was utilized (Weinbaum & Burger, 1973) to investigate whether Ca^{2+}-promoted association between AF molecules could provide sufficient adhesive forces for cellular aggregation. Sepharose beads were covalently coupled to purified aggregation factor. The properties of such AF-coupled beads are shown in Figure 3. Under standard aggregation assay conditions, the beads aggregated spontaneously in response to Ca^{2+} and aggregation was enhanced by addition of exogenous AF.

The binding of [^{125}I]AF to AF-coupled beads was examined (Figure 4) to quantitate the effect of Ca^{2+} on AF–AF interaction. The Ca^{2+} dependence of AF–AF association closely resembled that of cell aggregation, suggesting a common mechanism.

Inhibitors of AF-Promoted Cell Aggregation: Effects on [^{125}I]AF Binding. Previous studies have shown *Microciona* aggregation factor to be irreversibly inactivated by such treatments as 10 mM EDTA, heat (50 °C, 10 min), periodate, and certain proteases (Moscona, 1968; Henkart et al., 1973; Cauldwell et al., 1973; Humphreys et al., 1977). The effects of some of these treatments on [^{125}I]AF binding to cells and to AF beads were compared to investigate the molecular basis of AF–cell interaction. The results shown in Table II indicate that heat, EDTA, and periodate treatments drastically reduce [^{125}I]AF binding to AF beads but have little effect on binding to cells. Control experiments show that the binding of inac-

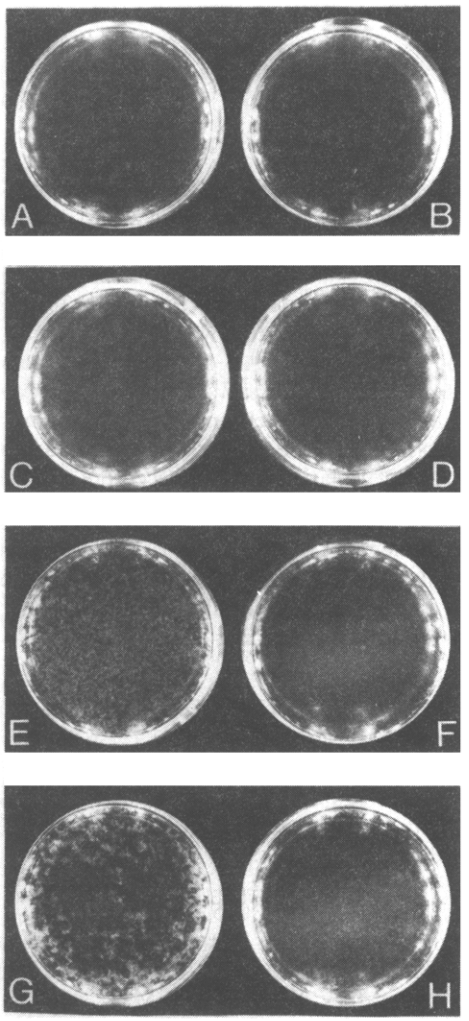

FIGURE 3: Aggregation of AF-coupled agarose beads in response to Ca^{2+} and exogenous AF. AF-coupled beads or BSA-coupled control beads were washed and diluted with CMFT ± 0.01 M Ca^{2+} to a bead concentration of $\sim 10^6$/mL. 0.4-mL portions of the bead suspensions were incubated in Linbro plates for 20 min under standard cell aggregation assay conditions (see Materials and Methods). Photographs were taken ~10 min after the 20-min incubation on the rotary shaker. (A) AF beads in CMFT; (B) BSA (control) beads in CMFT; (C) AF beads in CMFT + 4 units/mL exogenously added AF; (D) BSA beads in CMFT + 4 units/mL AF added; (E) AF beads in CMFT + 10 mM $CaCl_2$; (F) BSA beads in CMFT + 10 mM $CaCl_2$; (G) AF beads in CMFT + 10 mM $CaCl_2$ + 4 units/mL added AF; (H) BSA beads in CMFT + 10 mM $CaCl_2$ + 5 units/mL added AF.

tivated AF remains species specific (Table II). Thus, the inhibitory action of these treatments appears to be related to the Ca^{2+}-dependent secondary associations between AF molecules which function in cell aggregation.

The baseplate component, a soluble, receptor-like inhibitor of AF-promoted cell aggregation (Weinbaum & Burger, 1973; Jumblatt et al., 1977), was found to strongly inhibit binding of [^{125}I]AF to both cells and AF beads (Table II).

Discussion

In this paper we describe the first direct, quantitative binding studies of sponge aggregation factor to cells under the conditions of cell aggregation. Binding of *M. prolifera* aggregation factor appears to be highly species specific, as expected from its specificity in promoting homotypic cell aggregation (Humphreys, 1963; Moscona, 1968; Burger & Jumblatt, 1977; Table II), confirming earlier observations by Moscona that *Microciona* aggregation factor activity is selectively absorbed from solution during incubation with live or formaldehyde-

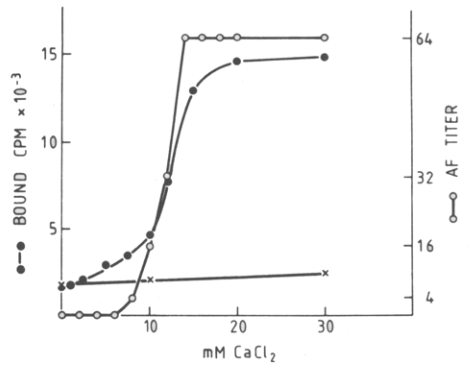

FIGURE 4: Ca^{2+}-dependent binding of [^{125}I]AF to AF-conjugated beads. AF-coupled beads or BSA-coupled control beads ($\sim 10^6$/mL) were incubated with ^{125}I-labeled aggregation factor (1 unit/mL, 2 × 10^6 Ci/mol) in CMFT supplemented with $CaCl_2$ at the concentrations shown. Following the 20-min incubation in the standard assay, beads with bound [^{125}I]AF were separated by centrifugation and counted (see Materials and Methods). The Ca^{2+} dependence of AF-promoted cell aggregation, shown previously in Figure 2, is shown again in this figure. (●) [^{125}I]AF bound to AF beads; (×) [^{125}I]AF binding to BSA (control) beads; (○) AF-mediated cell aggregation (AF titer) in response to the respective Ca^{2+} concentrations.

Table II: Effect of AF Pretreatment on Cell or Bead Aggregation Compared to the Binding to Cells and AF-Conjugated Beads

	pretreatment of *Microciona* [^{125}I] AF[a]	cell or bead aggregation after incubn with [^{125}I]AF	[^{125}I] AF bound[b] (cpm)
Microciona cells	none	+++	20 481
	50 °C, 10 min	+	18 024
	10 mM EDTA, 30 min	+	31 473
	5 mM periodate, 3 h	–	15 358
	baseplate, 2.2 μg/mL	+	5 650
Cliona cells	none	–	2 162
	50 °C, 10 min	–	1 324
	10 mM EDTA, 30 min	–	1 642
	5 mM periodate, 3 h	–	1 510
AF-conjugated beads	none	+++	25 919
	50 °C, 10 min	+	8 381
	10 mM EDTA, 30 min	+	9 014
	5 mM periodate, 3 h	+	5 501
	baseplate, 2.2 μg/mL	+	5 965
BSA-conjugated beads	none	–	2 537

[a] Following the pretreatments, the treated *Microciona* [^{125}I] AF was dialyzed against 1000 volumes of CaCMFT for 4 h to remove residual EDTA or periodate and then was used for binding assays. Where indicated, the [^{125}I] AF was incubated with baseplate (2.2 μg of protein per mL) for 10 min prior to addition of cells or conjugated agarose beads to initiate [^{125}I] AF binding. [b] [^{125}I] AF, pretreated as indicated, was incubated at 1 unit/mL with glutaraldehyde-fixed *Microciona* or *Cliona* cells (10^7 cells/mL) or with coupled agarose beads (10^6 beads/mL). After 20 min of incubation, cell- or bead-associated radioactivity was determined as described under Materials and Methods.

fixed cells of the same species (Moscona, 1968).

Results of binding measurements at the lowest active concentration of aggregation factor (1 unit/mL) indicate that as few as 400 molecules of AF bound per cell are sufficient to aggregate live or fixed cells in the standard assay. Based on a typical yield at 10 mg of purified AF obtained from 10^{10} dissociated sponge cells, one can calculate the average amount

of AF associated with each cell in vivo to be 10^{-12} g or 4.8×10^{-20} mol of 21×10^6 dalton monomer or ~28 000 molecules per cell. This apparent excess of aggregation factor in vivo raises the question of whether cell–cell adhesion is the primary function of AF in sponge tissue. Other suggested functions include a development role in cell sorting and pattern formation based on quantitative differences in cellular affinities for AF (Steinberg, 1970), a structural role analogous to mammalian cartilage proteoglycans (Hascall & Sajdera, 1969) as a component of the extensive extracellular matrix of sponge tissue, or a role in maintaining species integrity during gamete interaction (Burger, 1977) or invasive colonialization by other sponge species (Moscona, 1968). Although purified AF appears to be homogeneous, based on physical–chemical criteria, it may consist of a population of molecules or subunits with distinct tissue or cellular affinities. To approach such questions, we are currently comparing the binding of AF to purified cell types and investigating the histological and cellular distribution of bound AF by fluorescent antibody techniques.

The mechanisms of AF-promoted cell adhesion in *Microciona* involves two processes: (1) the binding of monomeric AF complexes to specific cellular sites and (2) the Ca^{2+}-dependent interaction between bound AF molecules to link adjacent cells. The apparent nonsaturability of AF binding to cells in normal seawater (Figure 1) may result from Ca^{2+}-mediated associations between bound and free AF molecules, as suggested by the AF-conjugated bead model (Figure 4). In support of this interpretation, saturable binding of AF to cells has been observed in divalent cation free seawater (J. Jumblatt and M. M. Burger, unpublished experiments). The finding that AF–AF interactions are sensitive to heat, EDTA, and periodate whereas AF binding to cells is unaffected by such treatments suggests that the two activities involve separate elements of the AF molecule. On the basis of size and structural complexity, one would expect the 21×10^6 dalton molecule to possess multiple active sites for interaction with cells. However, our results show the AF monomer to be functionally univalent, i.e., incapable of promoting stable intercellular linkages in the standard assay, at Ca^{2+} concentrations below that of normal seawater (~10 mM Ca^{2+}). Although direct bridging of cells by monomeric AF remains possible under lower shear forces or in vivo, the fact that the release of AF from freshly dissociated sponge cells is facilitated by removal of divalent cations suggests that a large proportion of cell-associated AF in the sponge is complexed with other AF molecules rather than cellular receptors.

The utilization of AF-conjugated agarose beads as a model for cell aggregation provides additional evidence that Ca^{2+}-mediated association between exposed AF molecules is sufficient to form stable intercellular adhesions. The kinetics and Ca^{2+} dependence of AF bead aggregation are strikingly similar to mechanically dissociated sponge cells, i.e., cells with a functional complement of AF molecules on their surfaces (Humphreys, 1963; Moscona, 1968). The precise role of Ca^{2+} in inducing AF–AF association remains unclear, although Ca^{2+} bridging seems a likely possibility. *Microciona parthena* AF has been found to contain a large proportion of acidic amino acids and saccharides and to bind several thousand additional Ca^{2+} ions at Ca^{2+} concentrations between 1 and 10 mM (Henkart et al., 1973; Cauldwell at al., 1973). Alternatively, Ca^{2+}-dependent conformational shifts in the AF molecule may be involved in the formation of higher order aggregates (Cauldwell et al., 1973). Preliminary attempts to reconstitute EDTA-inactivated AF have been unsuccessful, but such an approach may eventually elucidate the role of Ca^{2+} in AF structure and function.

The chemical nature of the interaction between the active site(s) on the AF molecule and its cellular receptor(s) remains a key question with regard to AF specificity. Previous results (Turner & Burger, 1973) suggested that specific saccharide residues on the AF molecule of *M. prolifera* may be recognized by lectin-like surface proteins, although we have so far been unable to inhibit binding of ^{125}I-labeled AF to cells by addition of a variety of hapten saccharides. In support of the receptor-like properties of the cell surface baseplate component described earlier (Weinbaum & Burger, 1973), a partially purified baseplate preparation was found to inhibit both the binding of AF to cells and AF–AF interaction, possibly by a related mechanism. The assay systems we have developed to distinguish between AF–cell and AF–AF binding will be a useful supplement to the cell aggregation assay for the remaining task of isolating cellular receptors and identifying active components of the AF molecule.

Acknowledgments

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